

AD _____

Award Number: DAMD17-01-1-0571

TITLE: Heat Shock Protein 27 Inhibits Apoptosis by Binding
Cytochrome c

PRINCIPAL INVESTIGATOR: Stephen W. Carper, Ph.D.

CONTRACTING ORGANIZATION: University of Nevada, Las Vegas
Las Vegas, Nevada 89154-1055

REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030214 125

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)**2. REPORT DATE**

June 2002

3. REPORT TYPE AND DATES COVERED

Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLEHeat Shock Protein 27 Inhibits Apoptosis by Binding
Cytochrome c**5. FUNDING NUMBERS**

DAMD17-01-1-0571

6. AUTHOR(S)

Stephen W. Carper, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)University of Nevada, Las Vegas
Las Vegas, Nevada 89154-1055**E-Mail:** carpers@unlv.edu**8. PERFORMING ORGANIZATION
REPORT NUMBER****9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**10. SPONSORING / MONITORING
AGENCY REPORT NUMBER****11. SUPPLEMENTARY NOTES****12a. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE**13. ABSTRACT (Maximum 200 Words)**

The purpose of this research project is to investigate the interaction between heat shock protein 27 (hsp27) and cytochrome c in the inhibition of apoptosis. The scope of the study will include: measuring the binding of hsp27 to cytochrome c in vivo, determining why hsp27 binds to cytochrome c and determining the fate of the hsp27:cytochrome c complex. Major findings to date include: cellular survival cures to cytochrome c and molecular chaperone assays with hsp27 and citrate synthase and alpha-glucosidase. A no-cost one year time extension was granted to complete the goals of the project.

14. SUBJECT TERMS

breast cancer

15. NUMBER OF PAGES

5

16. PRICE CODE**17. SECURITY CLASSIFICATION
OF REPORT**

Unclassified

**18. SECURITY CLASSIFICATION
OF THIS PAGE**

Unclassified

**19. SECURITY CLASSIFICATION
OF ABSTRACT**

Unclassified

20. LIMITATION OF ABSTRACT

Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	4
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	5

INTRODUCTION:

This annual report is for the period June 2001 – May 2002. The objectives of the purposed study are to (1) measure the binding of hsp27 to cytochrome c in vivo, (2) determine why hsp27 binds to cytochrome c and (3) determine the fate of the hsp27:cytochrome c complex. We have made significant progress on the stated objectives but have not yet achieved them, so we asked for and received a one year, no-cost, time extension to complete the objectives.

BODY:

The first objective, measure the binding of hsp27 to cytochrome c in vivo, is progressing well. We have made extracts of cells induced to undergo apoptosis. We have also successfully conducted Western blot analysis of in vitro immunoprecipitated samples of hsp27 and cytochrome c. We are now ready to begin the immunoprecipitation of the in vivo samples. To demonstrate that cytochrome c can interact with hsp27 in vivo, we have added cytochrome c to whole cells and determined that cells that constitutively express hsp27 survive at a much greater frequency then cells that did not express hsp27. We believe (and are in the process of demonstrating) that cytochrome c enters the cell and triggers apoptosis.

The second objective, determine why hsp27 binds to cytochrome c is in progress. We have begun using a DHFR refolding model and have determined the conditions that are necessary to thermally denature DHFR. We have also used citrate synthase and alpha-glucosidase as model systems as well.

The last objective, determine the fate of the hsp27:cytochrome c complex, is also showing good progress. We have initially focused on using a chromatography approach to help identify the hsp27:cytochrome c complex. We are using the apoptosome as a model system to determine if hsp27 can prevent its formation. We have determined the optimum conditions for the column and are now ready to begin a time course study.

KEY RESEARCH ACCOMPLISHMENTS:

Cellular Survival Curves to Cytochrome c.

Hsp27 Molecular Chaperone assays with Citrate Synthase and Alpha-Glucosidase

Determination of Column Conditions for Apoptosome Detection

REPORTABLE OUTCOMES:

Scientific talk "The role of heat shock proteins in the inhibition of apoptosis" given at the 2002 Radiation Research Society Meeting in Reno, NV that outlined our progress on this project.

Poster session "Heat Shock Protein 27 Inhibits Apoptosis in Human Breast Cancer cells by Binding to Cytochrome c" given at the 2002 Era of Hope U.S. Army Breast Cancer Research Conference in Orlando, Fl., that outlines our progress to date.

CONCLUSIONS:

More time is needed to achieve the objectives. We believe that we will be able to achieve the three goals by the end of the time extension. The data generated by these studies should be sufficient for two publications as well as a competitive grant application to the U.S. Army to extent these studies.

REFERENCES: None

APPENDICES: None